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The actin gelling activity of chicken gizzard α-actinin at physiological temperature is triggered by water sequestration

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At 37°C, in the presence of 6% (w/v) polyethylene glycol 6000, 30 nM α-actinin from chicken gizzard induces the gelation of 12 μM actin. Static measurement shows that the addition of 30 nM α-actinin increases the rigidity of the system from 23.5 to 54 dynes/cm². According to the theory of osmoelastic coupling, also large additives, such as the proteins of the cell sap, are able to cause an osmotic stress equivalent to that caused by polyethylene glycol. We thus conclude that, in vivo, α-actinin acts as an actin gelling protein.

α-Actinin; Physiological temperature; Water sequestration

1. INTRODUCTION

It is a common notion that increasing temperature decreases the effect induced by the actin gelling proteins, an effect which is almost undetectable at 37°C [1–5]. This behaviour, apparently, casts doubt on the functioning of these proteins as gelation factors in vivo [3].

Polyethylene glycol 6000 was reported to promote the formation of F-actin from G-actin [6] and, more recently, polyethylene glycol and ovalbumin were shown to cause the formation of parallel bundles of actin filaments under physiological conditions of ionic composition and pH [7]. These effects were explained by the theory of osmoelastic coupling, in which the elasticity of actin filaments is coupled with the osmotic stress due to the local imbalance of osmolarity that is caused by addition of a large additive such as polyethylene glycol or ovalbumin [7,8].

Since osmoelastic coupling favours the association between proteins, it is conceivable that it could favour also gelation at physiological temperature. This is indeed the case. We show that, at 37°C, in the presence of 6% (w/v) polyethylene glycol 6000, 30 nM α-actinin from chicken gizzard induces the gelation of 12 μM actin. A similar effect may be promoted by the proteins of the cell sap. We thus conclude that α-actinin acts as an actin gelling protein in vivo.

2. MATERIALS AND METHODS

G-Actin from rabbit muscle was prepared and assayed as previously described [9]. α-Actinin from chicken gizzard was prepared according to Feramisco and Burridge [10]. α-Actinin concentration was measured from the absorbance at 278 nm, the absorbance of 1 mg of pure α-actinin/ml (light path 1 cm) being taken to be 0.97 [11]. Molar concentrations were calculated on the basis of an M_r of 200 000 [11].

Viscosity was measured either with Ostwald viscosimeters (water flow time 60 s at 20°C) [12] or with home-made 'falling ball' viscosimeters (capillary diameter, 2.03 mm; capillary slope 22°) maintained in thermostatted water baths [13].

To measure the rigidity of the samples, the gels were allowed to form by incubating the actin-α-actinin solutions in graduated glass tubes (inner diameter 6 mm). The measurements were performed by delivering, under the meniscus of the solutions, by means of a Terumo microsyringe, 0.025 ml droplets of toluene–carbon tetrachloride mixtures of different density. Each tube was utilized for only one determination. The trials were continued until the density was found that allowed the droplet to remain stationary in the tube. The rigidity (T_o) was calculated according to the equation [13]:

$$T_o = 0.76 \times r \times \alpha \, (\varrho_d - \varrho_m)$$

where r is the radius of the droplet (0.182 cm), α is the acceleration due to gravity (980 $cm \cdot s^{-2}$) and ϱ_m and ϱ_d are the densities of the medium and of the droplets, respectively, in g/ml.

3. RESULTS

3.1. *In the presence of polyethylene glycol 6000, α-actinin promotes the gelation of F-actin even at 37°C*

α-Actinin (30 nM) does not affect the low shear viscosity of F-actin (12 μM as the monomer) at 37°C. However, when the system is supplemented with polyethylene glycol 6000 (PEG), a sharp increase of the low shear rate viscosity is observed. The phenomenon occurs beyond a critical PEG concentration (5–6%, w/v) and is concomitant with the PEG-induced transition of actin filaments into bundles of filaments [14].

At lower PEG concentrations (1–4%) the low shear rate specific viscosities of F-actin plus α-actinin and of F-actin alone are not significantly different from those

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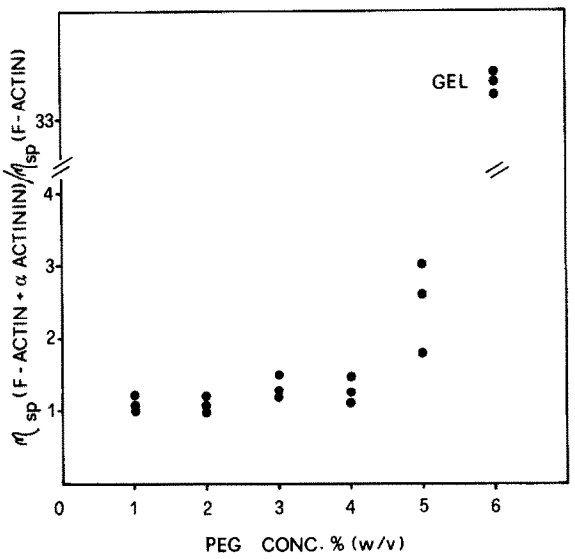

Fig. 1. Effect of α-actinin on the low shear rate viscosity of F-actin at increasing concentrations of polyethylene glycol. The mixtures contained F-actin (12 μM as the monomer), 0.2 mM ATP, 2 mM $MgCl_2$, 0.1 M KCl, 10 mM Tris-HCl buffer, polyethylene glycol 6000 (w/v) as indicated in the figure, with or without 30 nM α-actinin. After 90 min of incubation at 37°C and pH 7.5, in the tubes of the falling ball viscosimeters, the low shear rate viscosity of the samples was determined. Ordinate: ratio of the low shear rate specific viscosities of the F-actin-α-actin mixture and of F-actinin alone.

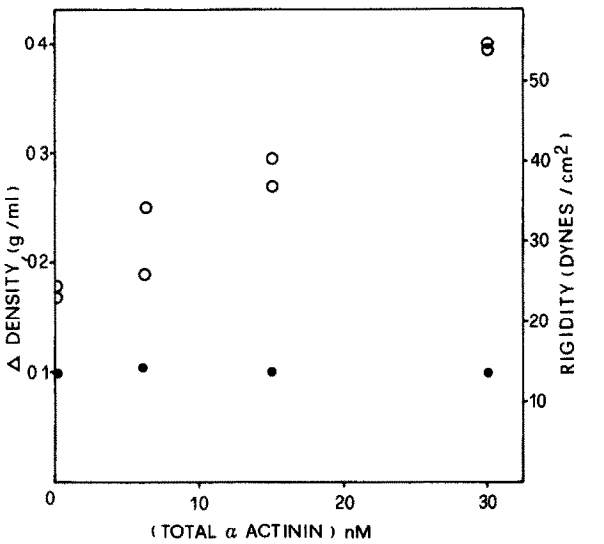

Fig. 2. Estimate of the effect of increasing α-actinin concentrations on the rigidity of the network formed by F-actin in the presence and in the absence of polyethylene glycol. The mixtures contained F-actin (12 μM as the monomer), 0.2 mM ATP, 2 mM $MgCl_2$, 0.1 M KCl, 10 mM Tris-HCl buffer with (○), or without (●) 6% (w/v) polyethylene glycol 6000. Concentration of α-actinin was as indicated in the figure. After 90 min of incubation, at 37°C and pH 7.5, in glass tubes (inner diameter 6 mm), rigidity measurements were performed as described in section 2. Δ-Density represents the difference between the density at which the droplets remained stationary in the complete system and in the salt solution without protein.

obtained in the absence of PEG and their ratios oscillate between 1.1 and 1.5 (Fig. 1). When the concentration of PEG is increased to 6%, the low shear rate viscosity of F-actin alone increases 5 times, as the average, with respect to the values obtained in the absence of PEG, while the F-actin-α-actinin mixture undergoes gelation. As a consequence, the ratio of the low shear rate viscosity of the two systems becomes very high (>33) (Fig. 1).

3.2. *In the presence of polyethylene glycol 6000, α-actinin increases the rigidity of the F-actin network even at 37°C*

In the falling ball viscosimeter, the rate of shear decreases with the increase of the viscosity of the samples. This leads to overestimation of the viscosity of the more viscous samples as compared to the viscosity of the less viscous samples [15]. To eliminate these artifacts we have performed static measurements of the rigidity of the F-actin-α-actinin and of the F-actin-α-actinin-PEG systems. At 37°C, the rigidity of F-actin (12 μM as the monomer) was 13.5 dynes/cm^2 and was not modified by the addition of α-actinin (Fig. 2). In the presence of 6% PEG, the rigidity of F-actin (12 μM as the monomer) was 23.5 dynes/cm^2 and increased to 54 dynes/cm^2 in the presence of 30 nM α-actinin (Fig. 2). It was, therefore, confirmed, also by static measurements, that α-actinin, in the presence of PEG, increases the rigidity of the F-actin network.

4. DISCUSSION

We have found that polyethylene glycol 6000 allows α-actinin to act as an actin-gelling protein even at physiological temperature. Since any macromolecule is able to induce an osmotic stress by sequestration of water [7,8], proteins of the cell sap too must be able to support the gelling activity of α-actinin. Our observation, therefore, proves that the proteins of the α-actinin family are efficient actin gelling agents in vivo.

Very likely they display their action by cross-linking bundles of actin filaments, thus creating a strong network. This overcomes one of the causes of the collapse of a gel; the spontaneous fragmentation of actin filaments [16], which, certainly, increases with thermal motion.

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